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THE QUEENSLAND JOURNAL OF AGRICULTURAL SCIENCE

VOL. 12. - - NO. 3.

SEPTEMBER, 1955.

THE SEED FAT OF *MALLOTUS PHILIPPINENSIS* (KAMALA OIL)—AN EXAMINATION OF SOME AUSTRALIAN MATERIAL.

By N. C. HANCOX, M.Sc., and H. H. HATT, D.Sc., Ph.D., Division of Industrial Chemistry, Commonwealth Scientific and Industrial Research Organization.

SUMMARY.

Australian samples of seeds of Mallotus philippinensis, a tree which has recently received much attention in India as a source of a seed oil possessing drying properties very similar to those of tung oil, have been examined.

The Australian oil is shown to be comparable with the Indian.

Obstacles to economic production are harvesting difficulties, smallness of the fruit, variability in the quality of the oil, and the need to use solvents other than the usual light petroleum for extraction.

Mallotus philippinensis, which belongs to the family Euphorbiaceae, is a tree distributed widely over South-East Asia and also occurring in Australia (coastal Queensland and northern New South Wales). In India, it has long been of minor economic value because of the red dye "kamala" with which the fruits are coated. Recently much attention has been paid there to the seed fat termed "kamala oil," which has powerful drying properties like those of tung oil (Aggarwal, Bhatnagar, Narain and Karimullah 1948; Sharma and Aggarwal 1952). These are due to the presence, as major constituent acid, of kamlolenic acid which like elaeostearic acid of tung oil has a system of three conjugated double bonds, and differs from it only in the possession of a terminal hydroxyl group (Gupta, Sharma and Aggarwal 1951; Calderwood and Gunstone 1953; Crombie and Taylor 1954).

In view of the wide Australian occurrence of *M. philippinensis* (and several other *Mallotus* species), and the fact that it is one of the very few native plants known to produce a drying oil, it was considered highly desirable to examine some material of local origin and compare it with Indian material.

It is obvious that for commercial exploitation to be feasible many problems must first be overcome, not the least of which are those calling for botanical study. The tree is said to grow to a height of 50 ft., but is often small

The kamlolenic acid of *M. philippinensis* has been thoroughly investigated from a structural point of view by other workers (Sharma and Aggarwal 1952; Calderwood and Gunstone 1933; Crombie and Taylor 1954). It differs from elaeostearic acid only in possessing a terminal hydroxyl group. This hydroxyl group appears to have no adverse effect on the drying properties; indeed, the most striking difference of the fat from tung oil is the much shorter gelation time at 280°C. in the Browne Heat Test (tung oil 9–12 min., kamala < 2 min.). The hydroxyl group does have the undesirable effect of raising the melting point of the fat above room temperature. Its presence, however, is responsible for a profound difference between the constitution of the fat itself and that of tung oil and all other fats.

Work at the Paint Research Station of Teddington, England (O'Neill, Dennison and Ahlers 1954) has shown that kamala oil has only one-third of the expected glycerol content of a fat and cannot therefore have a normal triglyceride structure. Instead the kamlolenic acid must be present wholly or in part in the form of a polyester, in which its hydroxyl groups have taken over the functions which those of glycerol perform in a normal fat. This is a feature unique in fat chemistry. The present work confirms O'Neill's findings of abnormally low glycerol content: we found values of 2·8% to 4·8%.

The fact that kamlolenic acid has this terminal hydroxyl group and is prone to form polyesters may well open up a wider field of use than merely as a replacement for tung oil. For example, it provides a source of long-chain hydroxy or dibasic acids of a type otherwise difficultly accessible.

The development of these industrial potentialities obviously depends on the supply of material being adequate, the quality uniform, and the price within reasonable bounds. The fat does not appear to be yet in commercial production in India, where the position is probably more favourable because of lower labour costs and the fact that there is a small industry based on kamala dye. In Australia it would seem that the botanical problems outlined earlier in this note are those which need attention before commercial possibilities could reasonably be assessed. The chemical merits of the material seem sufficient to make such a botanical study worth while.

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FOETAL MALDEVELOPMENT IN A LITTER OF LARGE WHITE PIGS.

By J. W. RYLEY, B.V.Sc. (Senior Husbandry Officer, Animal Research Institute, Yeerongpilly), E. L. MELVILLE, Q.D.D. (Senior Adviser, Pig Branch), and J. S. F. BARKER, B.Agr.Sc. (Assistant Husbandry Officer, Animal Research Institute, Yeerongpilly).

SUMMARY.

Congenital deformities in a litter of Large White piglets, characterized by "kinky tails," cleft palates, hare lips, split ears, deformed limbs, supernumerary digits, and urogenital defects, are described and illustrated.

Breeding work to determine the cause of the deformities was not conducted, but it is suggested that they have a complex genetic basis that may involve a threshold during development.

I. INTRODUCTION.

Congenital abnormalities of man and animals are well known. In the pig, Buchanan Smith, Robinson and Bryant (1936) recorded a total of 21 abnormalities believed to be genetic defects, while Lerner (1944) recorded nine genetic lethals. Foetal maldevelopment in pigs believed to be of nutritional origin has been described by Hale (1935), and Ross, Phillips, Bohstedt and Cunha (1944).

During an experiment on the effect of *Leptospira pomona* infection on the breeding performance of sows (Ryley and Simmons 1954), one of the uninfected control sows farrowed a litter showing "kinky tail," cleft palate and a number of other anatomical defects. The details are reported in this paper.

II. HISTORY.

Ten purebred sows and a boar AHS of the Large White breed were obtained from a stud piggery in southern Queensland. Eight were maiden sows, but the other two had each produced one litter showing no deformities. Three of the sows had slightly deformed tails, but although the possibility of "kinky tail" was considered, it was decided that injuries were probably responsible.

III. OBSERVATIONS.

All 10 sows were mated to the boar AHS. One of them (Sow 11), which herself had a normal tail, produced a litter of 11 piglets all of which had "kinky tails." In eight of these piglets, there were also other anatomical defects—cleft palates, hair lips, split ears, deformed limbs and urogenital abnormalities, as shown in Table 1. Three of the piglets, including a mummified foetus, were born dead or died within a few minutes of birth. The birthweights of all the piglets are shown in Table 1. The mean weight of the eight living piglets was

1 lb. 15 oz., which is satisfactory for a first litter. Three (Numbers 1, 2, and 3) were destroyed five hours after birth because their hind limb deformities and cleft palates prevented their moving and suckling. Two (Numbers 7 and 8) lost weight rapidly and died within three days because of their inability to suckle. The other three (Numbers 9, 10 and 11), which had no visible abnormality except "kinky tail," survived and were weaned at eight weeks.

Table 1.

SEX, BIRTH WEIGHTS, SURVIVAL AND DISTRIBUTION OF ANATOMICAL ABNORMALITIES IN A LITTER OF ELEVEN LARGE WHITE PIGLETS.

Piglet No.	Sex.	Birth Weight.	Survival.	Kinky Tail.	Cleft Palate.	Hare Lip.	Split Ears.	Deformed Limbs.	Super-numerary. Digits.	Urogenital Abnor-mality.
		Lb. oz.								
1	F	2 4	Destroyed ..	+	+	+	+	+	+	—
2	F	1 15	Destroyed ..	+	+	+	+	+	+	+
3	F	1 8	Destroyed ..	+	+	+	+	+	+	—
4	M	1 12½	Born dead ..	+	+	+	—	+	+	+
5	F	0 14½	Born dead ..	+	+	+	+	—	+	—
6	..	0 4	Mummified ..	+	+	+	+	+	+	—
7	F	1 9	Died	+	+	+	—	—	—	—
8	M	2 2	Died	+	+	+	—	—	—	—
9	M	2 2	Weaned ..	+	—	—	—	—	—	—
10	M	2 3	Weaned ..	+	—	—	—	—	—	—
11	M	1 14	Weaned ..	+	—	—	—	—	—	—
			Totals	11	8	8	5	5	6	2

Key: .. = Mummified foetus, sex not determined.
+ = Abnormality present.
— = Abnormality absent.

The other nine sows, including three with deformed tails, produced litters with no visible anatomical defects. These sows included a litter mate of Sow 11 (Sow 3) which also had a normal tail. The boar AHS did not show any defects. Four of these sows, including the litter mate of Sow 11, were remated to the same boar and again all produced normal litters. Sow 11 was remated to a Tamworth boar and farrowed a litter that did not show anatomical defects.

As cleft palates, hare lips, polydactyly, kinky tail and defects of the ears in swine have been recorded either separately or in various combinations (Buchanan Smith *et al.* 1936), the pedigrees of Sow 11 and the boar AHS were examined. These pedigrees are presented in diagrammatic form in Fig. 1. Boar "E" is the grandsire of the boar AHS through both maternal and paternal lines, and grandsire of the sow through the paternal line. The coefficient of relationship between Sow 11 and the boar is 14%, while the inbreeding coefficient of the deformed litter is 7·4%.

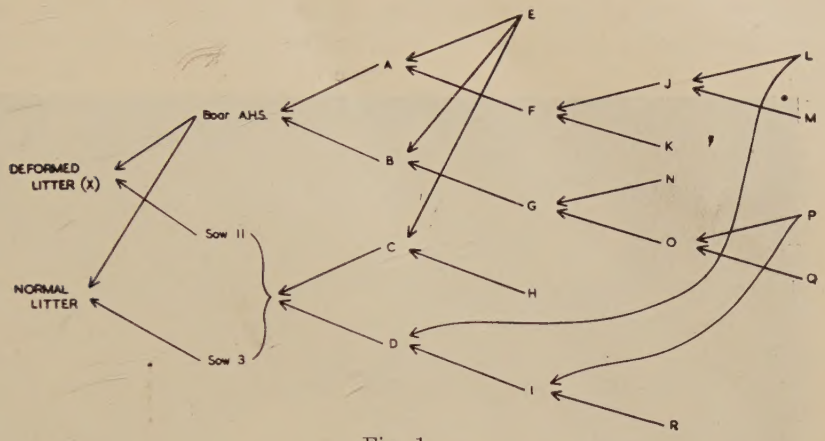

Fig. 1.
Diagrammatic Pedigree of the Litter of Deformed Large White Pigs.

IV. DESCRIPTION OF DEFORMITIES.

The deformities in each piglet are listed in Table 1 and four of the piglets are illustrated in Fig. 3.

"Kinky tails."—All 11 piglets had abnormalities of the tail varying from short thickened tails bent dorsally and anteriorly over the back to tails of normal length and thickness but bent at angles. X-ray photographs showed that these abnormalities were associated with either a reduction in the number of coccygeal vertebrae or a fusion of some of these vertebrae (Fig. 2).

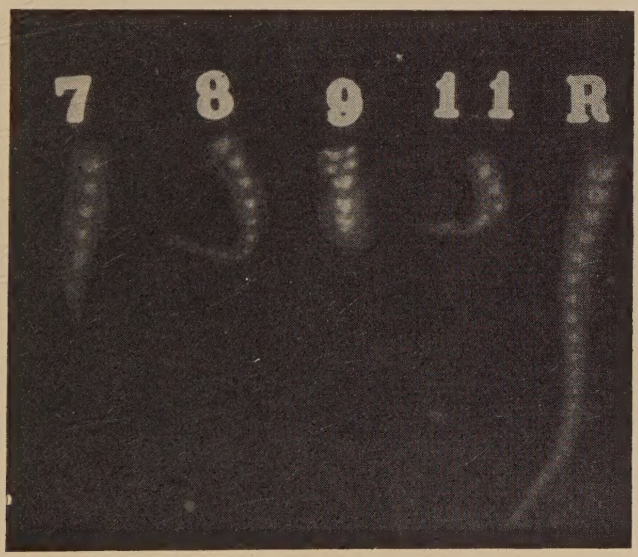

Fig. 2.

X-ray Photograph of the Tails of Piglets 7, 8, 9 and 11, Showing Reduction in Number and Fusion of Coccygeal Vertebrae Compared With Normal Tail "R" From a Newborn Piglet in a Normal Litter.

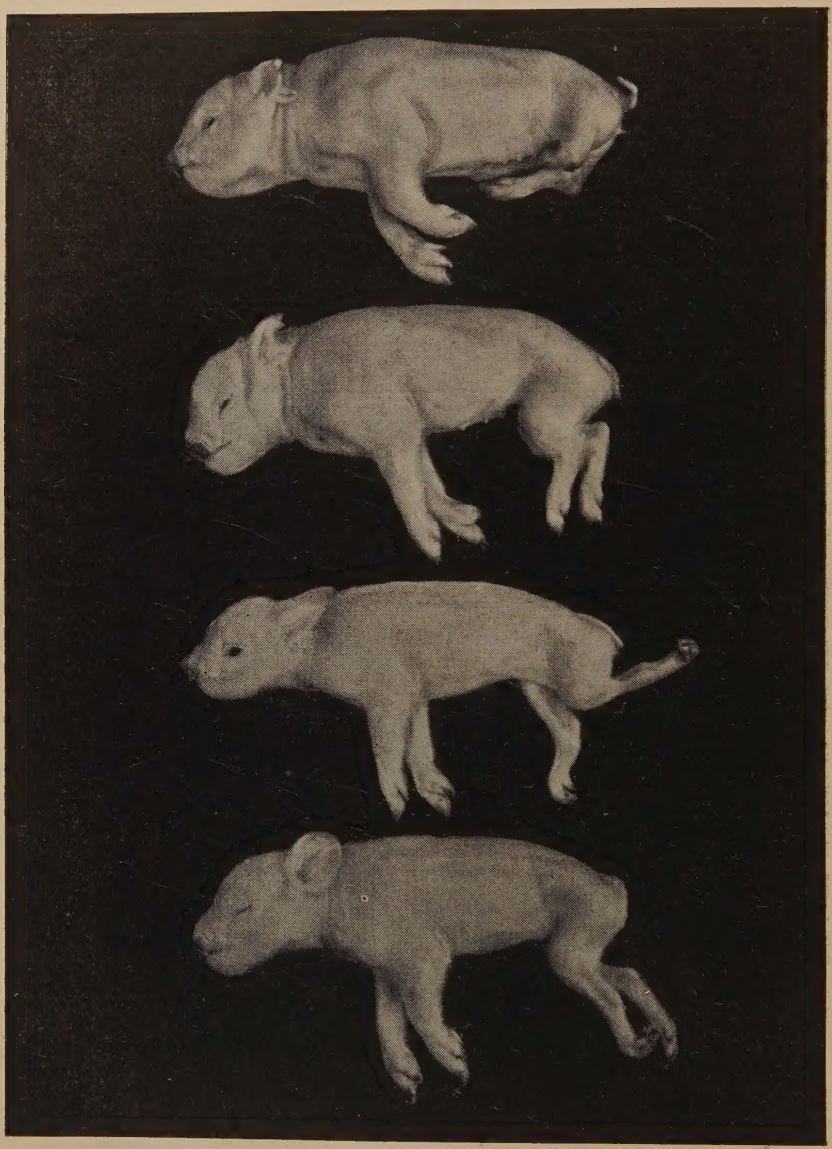

Fig. 3.

Piglets 1, 2, 3 and 4 (from top to bottom) Showing "Kinky Tail," Split Ears, Deformed Limbs and Supernumerary Digits.

Cleft palates.—Eight of the piglets had cleft palates. These clefts were irregular, usually on both sides of the raphe and varying from 0·5 in. to 3·0 in. long. This abnormality is shown in Fig. 4. In one piglet (Number 8) the cleft was unilateral.

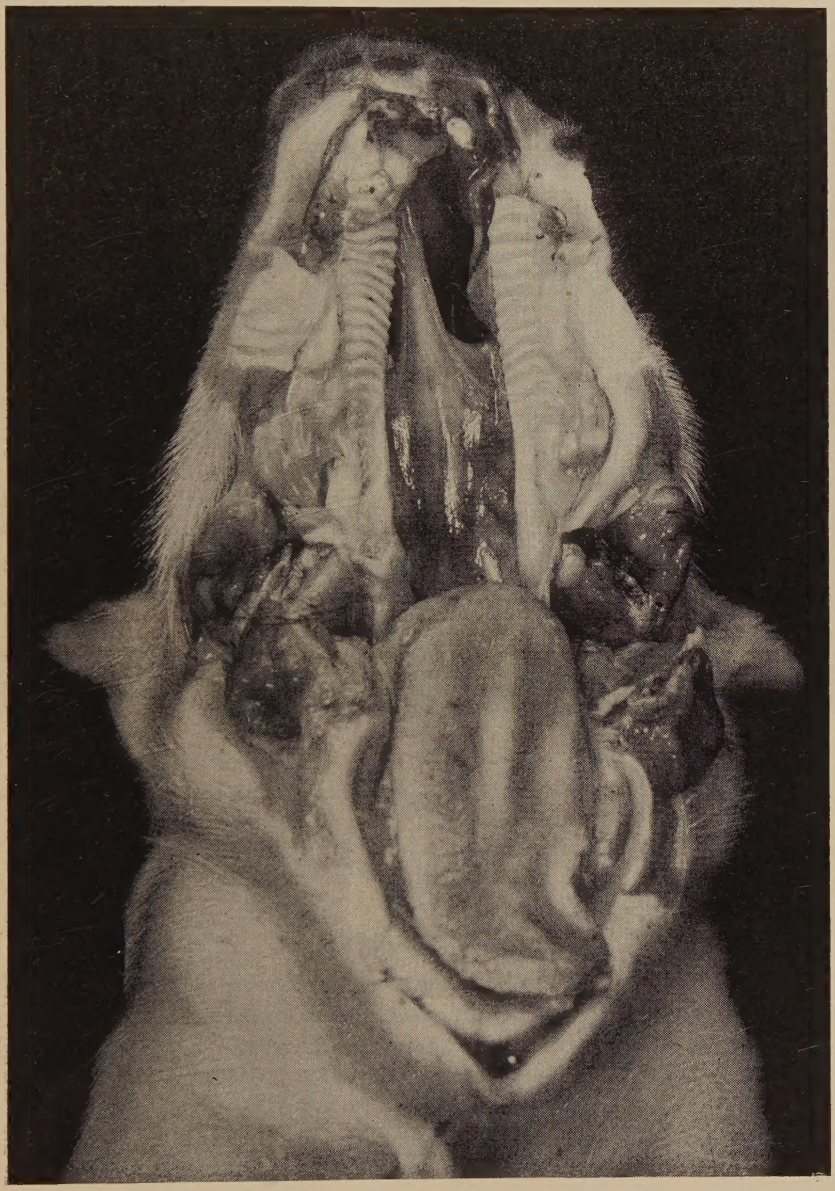

Fig. 4.

Dissected Buccal Cavity of Piglet 1, Showing Bilateral Cleft Palate

Fig. 5.

Unilateral Hare Lip and Distorted Incisor Teeth in Piglet 1.

Hare lips.—All eight piglets with cleft palates also had hare lips. The cleft in the lips extended to the nostrils on both sides except in Piglet 1 (Fig. 5), where it was unilateral. The incisor teeth were large and distorted.

Deformed limbs.—Four piglets showed deformities of the hind limbs. In Piglet 1, the hind limbs were bent under the abdomen. It was able to move, but had little control due to inability to stand on the hind limbs. Piglet 2 had an antero-medial twist in the stifle joint, and in Piglet 3 there was over-extension of the left stifle joint and marked flexion of both tarsal joints. Piglet 4 showed flexion of both the stifle and tarsal joints of the left limb and over-extension of the right tarsal joint.

Supernumerary digits.—Six piglets had supernumerary digits; in five the forelimbs were involved and in one (the mummified foetus) the hind limbs. These abnormalities in Piglets 1 and 2 were bilateral, Piglet 1 having a

supernumerary digit on the medial aspects while in Piglet 2 the medial digit was partially bifid. The abnormalities in Piglets 3 and 4 were present in the right forelimb only, Piglet 3 having a partly bifid medial digit and Piglet 4 a supernumerary digit on the medial aspect. Piglet 5 had two feet on both forelimbs and the mummified foetus (Number 6) had two feet on both hind limbs.

Split ears.—Five piglets had bilateral split ears as shown in Piglets 1, 2, and 3 in Fig. 3.

Urogenital defects.—Two of the piglets showed urogenital defects. Piglet 2 had two small kidneys on the left side joined by a common ureter but with no connection between the parenchymatous tissue. The kidney on the right side showed no abnormality. Piglet 4 had one testis near the posterior pole of the right kidney and the other partly down the inguinal canal.

V. DISCUSSION.

Foetal maldevelopment such as cleft palates, hare lips, malformed hind limbs and blind and eyeless piglets has been associated with vitamin A deficiency of brood sows during the first 30 days of gestation (Hale 1935). However, all sows in this experiment (Ryley and Simmons 1954) were fed a ration containing 5,000 I.U. of vitamin A per lb. and 2-3 lb. per day of freshly cut lucerne for two weeks prior to mating and during the whole gestation period. In addition, none of the piglets showed any abnormalities of the eyes, whereas 42 piglets born during the experiments of Hale (1935) were all blind. Some had no eyes, some had one and others had one large and one small eye. It is therefore unlikely that vitamin A deficiency *per se* was responsible for the abnormalities we observed.

Other nutritional deficiencies have been associated with congenital abnormalities in laboratory animals. Warkany, Roth and Wilson (1948) described shortening of limb bones, syndactylism and cleft palate in rats fed insufficient riboflavin. Gross abnormalities in rat embryos associated with pantothenic acid deficiency have been described by Lefebreres-Boisselot (1951, quoted by Hogan 1953). Ross *et al.* (1944) described congenital malformations in pigs such as fused toes, club feet, syndactylism, paralysis agitans, vestigial limbs, and "kinked tail." As the addition of good quality lucerne meal to the basal ration prevented their occurrence, they concluded that the abnormalities were of nutritional origin. None of these abnormalities, except "kinked tail," was seen in the litter described in this paper, and in addition the sow's ration included freshly cut lucerne. As the other nine sows mated to the boar AHS produced litters showing no defects, it seems unlikely that a nutritional factor *per se* was responsible.

Annett (1938) described cleft palates, split ears and deformed hindquarters in two of a litter of 14 purebred Tamworth piglets. A photograph in that paper shows a piglet similar to one depicted in Fig. 3. He considered that the defects were genetic in origin. As three of our original 10 sows showed "kinky tails" there may be some hereditary factor for these deformities carried in the parent stud. At the time the deformed litter was observed, it was not

possible to continue breeding in an attempt to determine the genetic basis, if any, of these deformities. The coefficient of inbreeding of the deformed litter is 7·4%. This is not a high coefficient, and even assuming simple inheritance of the defect, the chance that all 11 pigs of the litter would be affected is very remote.

In a series of Large White litters studied by Donald (1949), one litter was grossly deformed and contained piglets with abnormal tails, hind limbs, snouts, digits and urogenital organs. In addition, the "kinky tail" piglets in the other litters he studied showed a high incidence of abnormalities of the kidneys, ureters and genitalia. He concluded that the "kinky tail" character is genetic in nature, and the genes controlling it are of variable penetrance and expressivity. He thought that, in the Large White inbred strain studied, there were minor genes with a recessive effect as well as a major gene with incomplete dominance. Donald therefore suggested the hypothesis that pigs, in general, may be characterised by a slight tendency to the "kinky tail" defect, which is exhibited when the appropriate developmental accidents occur.

As all 11 piglets in the litter described in this paper were affected, it seems likely that the genetic basis is complex and that a "threshold" may be involved. During the pregnancy that produced the abnormal litter, some developmental accident could have occurred, allowing the developmental path to cross this threshold from the normal to the abnormal. As there are no breeding data other than the occurrence of this one defective litter, and as the developmental genetics of the pig have not been investigated, it is not possible to speculate further.

VI. ACKNOWLEDGEMENT.

We are indebted to the Photographic Section of the Queensland Department of Agriculture and Stock for taking the photographs of the deformed piglets and to Mr. S. Gould, M.I.R., of the University of Queensland Veterinary School, for his preparation of the X-ray photograph of the "kinky tails."

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STUDIES ON SEROLOGICAL DIFFERENTIATION OF HEATED ANIMAL PROTEINS.

By L. TAMMEMAGI, B.V.Sc., Dr.med.vet., Animal Research Institute, Yeerongpilly.

SUMMARY.

The technique of preparing antisera against heat-alkali-treated serum-antigens is described together with the elimination of cross-reacting "heat"-antibodies from such antisera.

The prepared antisera showed a rather low potency against normal sera but possessed a high sensitivity against serum-antigens heated from 70° to 100°C.

The absorbed (specific) antisera, however, were less satisfactory for differentiation of NaOH-extracted "insoluble" (boiled and powdered) serum-proteins than the unabsorbed (non-specific) antisera, and both failed completely to give precipitin reactions with cooked meat-proteins.

I. INTRODUCTION.

Several attempts have been made in the past to prepare antisera which could be used to identify heated animal proteins, such as horse meat, in processed goods (see below). Precipitating antisera obtained by immunizing rabbits with normal blood serum have been found of little value for identifying cooked meats that have been heated above 80° C. (Fornet and Mueller 1910, Proom 1943, Tammemagi 1954a).

Using heat-treated serum-antigens for immunization, several workers have prepared antisera which reacted either with heated only (Schmidt 1912) or with both heated and normal blood sera (Bruynoghe 1924, Meissner 1926) but not with muscle protein which had been rendered insoluble by heat (Schmidt 1912).

Apparently only Rosenberg (1926) has been able to produce antisera which would react with heat-denatured ("insoluble") muscle protein. She immunized rabbits with "heat-alkali" treated antigen of Schmidt (1912) or "heat-coagulated"-serum-antigen of Fujiwara (1922).

More recently Proom (1943) used extracts of boiled minced meat for immunization. The antibody response in the rabbit, however, was very poor, and the low titred sera obtained were non-specific.

Since no information could be found that Rosenberg's (1926) results have been reproduced, this paper describes experiments with antisera, produced by the method of Schmidt, as used by Rosenberg, for differentiating heated animal proteins.

II. MATERIAL AND METHODS.

(1) Preparation of Antisera.

(*a*) *Antigen for immunization.*—The method of Schmidt (1912) was used to prepare a "heat-alkali"-precipitogen for immunization of rabbits: 60 ml. normal saline solution was added to 60 ml. of serum and heated 30 minutes at 70°C. in a waterbath. After adding 10 ml. of N/1 NaOH, the solution was heated again 15 minutes at 70°C., by which time the previously viscous and turbid solution became clear and liquid.

(*b*) *Method of immunization.*—Six injections of 10 ml. of this antigen were given intramuscularly at weekly intervals into rabbits. The initially clear antigens obtained from pig and horse serum developed a slight deposit after a week's storage at 4°C. The ox and sheep antigens, however, developed a rather heavy deposit and became strongly opalescent. Before inoculation, the antigens were thoroughly shaken to resuspend this deposit.

Two rabbits were immunized with each of the four antigens. They were bled 2 weeks after the first inoculation, and thereafter before injecting each subsequent dose of antigen.

(*c*) *Technique of absorption.*—To eliminate cross-reacting antibodies from the antisera, the method of Weitz (1952) was used in a titration test to determine the proportion of heterologous heated antigens required to absorb the corresponding "heat"-antibodies. The antigen for the titration test was prepared by diluting normal serum 1/10 with saline solution, and then heating at 70°C. for 30 minutes, this dilution being necessary as at lower dilutions, or when undiluted, the serum became during heating either too gelatinous (dilutions 1/1—1/3), or too turbid (dilution 1/5) to be of any use.

(2) Precipitin Test.

The ring-precipitin test described in an earlier paper (Tammemagi 1954a) was used to determine the specificity and titre of the antisera against normal or heated serum and muscle proteins.

The time limit for reading a positive reaction was 2 hours at room temperature, but when negative after this time the final reading was done after incubating for 5 hours or longer at room temperature.

To determine the titres against heated serum-antigens, the homologous and heterologous normal sera were first diluted with saline to 1/10 and then heated for 30 minutes in a waterbath at various temperatures, viz. 70, 80, 90 and 100°C.

(3) Preparation of Heated (Insoluble) Proteins for Testing.

(*a*) *Heated serum-proteins.*—The method of Schmidt (1912) was used to prepare heated (insoluble in indifferent solvents, i.e. water, saline etc.) serum-proteins for identification tests:

Undiluted blood serum was heated for 3 hours in a boiling waterbath. To remove all traces of soluble protein particles, the coagulum was thoroughly washed with saline, dried overnight at 37°C. in an open petri dish and pulverised. A heaped knife-point-full of the powder was shaken with 10 ml. of N/10 NaOH, and heated for 10 minutes in a waterbath at 70°C. Some of the water-insoluble protein dissolves in the NaOH solution. To minimize the destructive effect of the warm NaOH, the solution was then poured into a flask containing 10 ml. of cooled saline solution. After filtering through paper, it was neutralized with N/20 HCl.

In Schmidt's original technique the neutralization point was a slight pinkish coloration of the solution with phenolphthalein as indicator. In these experiments, however, pH indicator-paper-slips were used to reach a slight alkaline reaction (about pH 7·5). Over-neutralization with HCl to neutral or acid reaction will cause a sudden turbidity. Addition of NaOH reverses this, and brings the proteins back into solution. According to Schmidt (1912) this manipulation does not interfere with the ultimate results.

(*b*) *Heated meat-proteins.*—Finely cut raw meat samples from various animal species were placed in screw-capped jars and heated in a boiling water-bath for from 30 minutes up to several hours. The juice that appeared during the boiling was collected and tested separately.

Portion of the meat residue was extracted with an equal weight of saline for one to five days, and the extracts, after being clarified through Whatman No. 1 filter papers or Zeiss coarse filter pads, examined serologically.

Another portion of the meat residue was treated in the same way as serum—i.e., dried at 37° C., powdered, extracted with NaOH and neutralized with HCl. However, treatment with NaOH produced very turbid filtrates, apparently due to fats being taken into solution by NaOH. Fat was eliminated eventually from the heated meat with chloroform which was changed several times during the day, and when necessary left to act over-night until no greasy flecks were left on filter paper.

III. RESULTS.

(1) Antibody Response to "Heat-alkali"-antigen.

(*a*) *Antibody titres against normal sera.*—From Table 1 it is evident that antisera produced by the use of "heat-alkali"-antigens gave positive reactions with normal sera, but the titres were surprisingly low compared with earlier results (Tammemagi 1954a) when normal or alum-precipitated antigens were used for immunization.

(*b*) *Antibody titres against heated sera.*—Compared with results obtained against normal sera (Table 1), the "heat-alkali"-antisera showed much higher titres against heated sera (Table 2). In some instances the highest homologous

Table 1.

PRECIPITIN TITRES OF ANTISERA, PREPARED BY INOCULATION OF RABBITS WITH SIX INTRAMUSCULAR INJECTIONS OF "HEAT-ALKALI"-TREATED ANTIGENS, WHEN TESTED WITH HOMOLOGOUS AND HETEROLOGOUS NORMAL SERA.

Antiserum.	Titres *Against Normal Sera of —			
	Horse.	Ox.	Sheep.	Pig.
Anti-horse	**100**	5	N	20
Anti-ox	N	**2,000**	200	20
Anti-sheep	10	500	**500**	50
Anti-pig	N	500	100	**500**

* Titres are expressed as reciprocals of dilution. Homologous titres are shown in black.

N indicates that a positive reaction appeared with undiluted ("Neat") normal serum only.

Table 2.

PRECIPITIN TITRES OF ANTISERA AGAINST HOMOLOGOUS AND HETEROLOGOUS SERA HEATED AT VARIOUS TEMPERATURES.

Heated Antigen.	Titres* of Antisera.			
	Anti-horse.	Anti-ox.	Anti-sheep.	Anti-pig.
Horse serum heated at 70°C.	**32,000**	8,000	8,000	16,000
Horse serum heated at 80°C.	**64,000**	8,000	4,000	32,000
Horse serum heated at 90°C.	**126,000**	8,000	4,000	—(4,000)
Horse serum heated at 100°C.	**32,000**	8,000	4,000	—(2,000)
Ox serum heated at 70°C.	8,000	**16,000**	4,000	16,000
Ox serum heated at 80°C.	8,000	**16,000**	4,000	16,000
Ox serum heated at 90°C.	16,000	**16,000**	8,000	?
Ox serum heated at 100°C.	16,000	8,000	4,000	?
Sheep serum heated at 70°C.	16,000	8,000	**32,000**	8,000
Sheep serum heated at 80°C.	16,000	8,000	**16,000**	16,000
Sheep serum heated at 90°C.	16,000	16,000	**16,000**	16,000
Sheep serum heated at 100°C.	16,000	8,000	**8,000**	4,000
Pig serum heated at 70°C.	16,000	4,000	4,000	**32,000**
Pig serum heated at 80°C.	16,000	4,000	4,000	**64,000**
Pig serum heated at 90°C.	16,000	4,000	—(4,000)	**32,000**
Pig serum heated at 100°C.	16,000	4,000	—(2,000)	**8,000**

* Titres are expressed as reciprocals of dilution. Homologous titres are shown in black.

? No determination possible because of interfering turbidity of the heated antigen dilution.

— No reaction after 2 hours of incubation at room temperature.

Figures in brackets indicate results after at least 4 hours of incubation.

or heterologous titres appeared with sera heated at 90°C., but usually the "heat"-antibody titres were about the same regardless of whether the antigens tested were heated at 70°, 80° or 90°C. Heating at 100°C, however, was generally followed by a decrease in the antibody reaction, and in some instances a ring-precipitation could be observed several hours after the usual time limit of 2 hours.

Increasing the temperature was usually followed by a progressively stronger opacity of the antigen solution. The reading of an homologous reaction was not greatly affected, as a ring-precipitation was distinct from the highest dilution (whatever the titre) down to a concentration of at least 1:100, or even to 1:10 of heated antigen. In heterologous reactions, however, where the ring formation was always weaker, the opacity of the antigen (especially when heated at 90° or 100°C.) frequently prevented detection of a positive reaction at a dilution lower than 1:1,000.

The ox serum when heated produced least opacity, followed in increasing order by horse, sheep and pig serum, where the turbidity was the greatest at any temperature of heating. Centrifugation or filtration through Whatman No. 1 filter paper did not clarify the turbid antigen (1/10) solutions. Filtration through Seitz bacteria-retaining filter pads produced perfectly clear filtrates, but apparently the antigen proteins were also removed from the filtrates as no precipitation reactions followed even in homologous reaction which was strongly positive prior to filtration.

(2) Absorption of Non-specific Antibodies.

From Table 2 it is seen that all four "heat-alkali"-antisera were non-specific, and except for anti-horse and anti-pig sera, there was no essential difference between the homologous and heterologous titres.

In attempts to obtain specific antisera against heated serum-antigens, a preliminary titration was done to determine whether heated heterologous antigens would absorb the corresponding "heat-alkali-precipitins" from the antiserum. For this purpose horse serum, diluted 1/10 and heated for 30 minutes at 70°C., was tested against horse antibodies in the anti-ox serum. The presence of any "normal" precipitins—that is, antibodies reacting only with normal, unheated serum-antigens—was regarded as unimportant.

Using Weitz (1952) technique for the titrations, it was observed that at the antigen (1:10)/antiserum ratio of 1/20 all horse antibodies ("Heat-alkali"-precipitins) apparently were removed as indicated by a negative precipitin reaction of the supernatant fluid in the corresponding tube when tested with 1:100 dilution of heated horse serum. Antigen solution 1:10 was unsatisfactory for testing because of a strong interfering turbidity at this dilution. The supernatant fluid still gave with sheep and pig antigens (heated) positive reactions even up to a dilution of 1:10,000 of these antigens, indicating that sheep and pig "heat-alkali-precipitins" were still present in the anti-ox serum after the absorption of the horse antibodies.

Since the heterologous antibody titres of all four antisera were about the same, it was considered that when increasing the ratio of the heterologous antigens in actual absorption from 1/20 to 1/10, it would be possible to absorb all cross-reacting antibodes (''heat-alkali-precipitins'') regardless of their titre.

In a first trial, simultaneous absorption with three different heterologous antigens caused the homologous antibodies to disappear. In further attempts, therefore, the absorption tests were done first with one single heterologous antigen, and if insufficient, two or more heterologous antigens were used simultaneously. The antigens in all these tests were heated at 70°C. (''70-degree group'').

Table 3.

RESULTS OF ABSORPTION OF ANTISERA WITH 1/10 VOLUME OF HETEROLOGOUS ANTIGENS, HEATED AT 70°C., WHEN TESTED AGAINST SERUM-ANTIGENS, HEATED AT EITHER 70°C. OR 80°C., AT DILUTIONS FROM 1/50 TO 1/1,000.

Antiserum.	Antigen.							
	Horse.		Ox.		Sheep.		Pig.	
	70°	80°	70°	80°	70°	80°	70°	80°
Anti-horse absorbed with Ox antigen	+	+	−	−	−	−	+	+
Anti-horse absorbed with Sheep antigen	+	+	−	−	−	−	+	+
Anti-horse absorbed with Pig antigen	+	+	+	+	−	−	−	−
Anti-horse absorbed with Ox and Sheep antigen	+	+	−	−	−	−	+	+
Anti-horse absorbed with Ox and Pig antigen	+	+	−	−	−	−	−	−
Anti-horse absorbed with Ox, Pig and Sheep antigen	+	+	−	−	−	−	−	−
Anti-ox absorbed with Horse antigen	−	−	+	+	+	+	+	+
Anti-ox absorbed with Sheep antigen	−	−	+	+	−	−	+	+
Anti-ox absorbed with Pig antigen	−	−	+	+	−	+	−	−
Anti-ox absorbed with Horse and Pig	−	−	+	+	−	+	−	−
Anti-ox absorbed with Sheep and Pig antigen	−	−	+	+	−	−	−	−
Anti-ox absorbed with Sheep, Pig and Horse antigen	−	O	−	O	−	O	−	O
Anti-sheep absorbed with Horse antigen	−	−	+	+	+	+	+	+
Anti-sheep absorbed with Pig antigen	+	+	−	−	+	+	+	+
Anti-sheep absorbed with Ox antigen	−	−	−	−	+	+	−	+
Anti-sheep absorbed with Horse and Pig antigen	−	−	−	−	+	+	−	+
Anti-sheep absorbed with Ox and Pig antigen	−	−	−	−	+	+	−	+
Anti-sheep absorbed with Horse, Ox and Pig antigen	−	O	−	O	+	O	−	O
Anti-pig absorbed with Horse antigen	−	+	−	+	−	−	+	+
Anti-pig absorbed with Ox antigen	+	+	−	−	−	−	+	+
Anti-pig absorbed with Sheep antigen	+	+	−	−	−	−	+	+
Anti-pig absorbed with Horse and Ox antigen	−	+	−	−	−	−	+	+
Anti-pig absorbed with Horse, Ox and Sheep antigen	−	+	−	−	−	−	+	+

\+ Positive reaction.
− Negative reaction.
O Not tested

The experiments (Table 3) showed that using any of the heated heterologous antigen alone at the ratio of 1/10, the corresponding antibody was absorbed from the antiserum, but in several instances it caused also the simultaneous disappearance of some other antibodies. Generally, however, it was necessary to use two antigens simultaneously to remove from each antiserum the three heterologous antibodies concerned. The use of three different antigens simultaneously caused the disappearance of the homologous antibodies from the anti-ox, but not from the other antisera.

In several instances, however, it was observed that antisera which apparently had become "specific" against antigens heated at 70°C. still gave cross reactions with one or other heterologous antigens heated at 80°C. To remove those antibodies which might react with antigens heated at this or higher temperature, absorption was repeated with antigens heated at 80°C. After this the antisera apparently had become truly specific, as they no longer reacted with any of the heterologous antigens heated either at 70, 80, 90 or 100°C. but would give definite homologous reactions regardless of the temperature applied to the antigen.

In the course of the experiments the antisera were rendered specific by selecting for absorption the following combinations of the heterologous antigens of the 80°C. type at the ratio of 1/10 to the antiserum:

anti-Horse serum by absorbing with Ox and Pig antigens;

anti-Ox serum by absorbing with Sheep and Pig antigens;

anti-Sheep serum by absorbing with Ox and Pig antigens;

anti-Pig serum by absorbing with Ox and Horse antigens.

After absorption the antisera were tested again with normal sera. In Table 4 it is shown that the antisera still gave cross reactions with heterologous "normal"-antigens, but a discrepancy is evident when comparing the results prior to absorption in Table 1.

Table 4.

PRECIPITIN TITRES OF ANTISERA AGAINST HOMOLOGOUS AND HETEROLOGOUS NORMAL SERA WHEN TESTED AFTER THE ABSORPTION WITH HEATED ANTIGENS.

Antiserum.	Titres* Against Normal Sera of—			
	Horse.	Ox.	Sheep.	Pig.
Anti-horse	**100**	—	—	20
Anti-ox	N	N	N	N
Anti-sheep .. .	N	100	**100**	50
Anti-pig	N	—	—	**500**

* Titres are expressed as reciprocals of dilution.

— Negative reaction, even with undiluted serum-antigen.

N indicates that a positive reaction appeared with undiluted ("Neat") normal serum only.

(3) Insoluble (Cooked) Serum-proteins.

Results of experiments with insoluble, pulverized serum coagulum of Schmidt, when extracted with NaOH and tested both with absorbed (specific) and unabsorbed (non-specific) antisera, are summarized in Table 5.

Table 5.

PRECIPITIN REACTIONS OF ABSORBED AND UNABSORBED "HEAT-ALKALI"-ANTISERA AGAINST NaOH-EXTRACTED INSOLUBLE (POWDERED) SERUM-PROTEINS.

Antiserum.	NaOH-extracted Serum-proteins of—			
	Horse.	Ox.	Sheep.	Pig.
Anti-horse, absorbed ..	+	−	−	−
Anti-horse, unabsorbed	+	−	−	−
Anti-ox, absorbed ..	−	−	−	−
Anti-ox, unabsorbed ..	+	+	+	−
Anti-sheep, absorbed ..	−	−	−	−
Anti-sheep, unabsorbed	−	−	+	−
Anti-pig, absorbed ..	−	−	−	+
Anti-pig, unabsorbed ..	−	−	−	+

Better results were obtained with unabsorbed than with absorbed antisera. All four unabsorbed antisera gave definite reactions with homologous antigens, but one of them, the anti-ox serum, also with horse and sheep antigens. Of the specific (absorbed) antisera only two, the anti-horse and anti-pig sera, gave homologous reactions, while the other two failed to do so.

(4) Meat Proteins.

In experiments with different meats, all the absorbed (specific) "heat-alkali"-immunsera failed to give homologous reactions with extracts prepared from minced raw or cooked meats even when the time of extraction was extended to 5 days. Similarly, negative results were obtained with juice that appeared during heating of meat samples in a boiling waterbath. Extracts, prepared by boiling minced raw meats with equal weights of saline for 1-3 hours failed also to show positive precipitin reactions.

Of the unabsorbed antisera, the anti-pig serum alone gave a positive precipitin reaction, and this only with meat juice, obtained by boiling pig meat in a waterbath for several hours.

Experiments with insoluble powdered cooked meats, when brought into solution by NaOH, all gave negative homologous reactions with both the absorbed and unabsorbed antisera.

IV. DISCUSSION.

The results of these experiments support the findings of Schmidt (1912) and Rosenberg (1926) that by using "heat-alkali"-antigens for immunizing rabbits, antisera can be prepared which are suitable for differentiation of blood sera which have been heated from 70° to 100°C.

Treatment of the antigen with NaOH for inoculation was introduced by Schmidt (1912) in order to obtain antisera which would react also with heated, insoluble proteins when the latter, for the purpose of precipitin test, are brought into solution by the use of NaOH. If rabbits are inoculated with "heat-precipitogen" (not alkali treated) only, the anti-sera ("Heat-precipitin") obtained would react with heated sera only so long as the serum-protein remains in the solution, or is soluble in indifferent solvents such as saline. When the protein has become insoluble after prolonged heating, a precipitin test is possible only when the protein is brought back into solution by a strong alkaline solution. By the action of alkaline, however, the protein undergoes such drastic changes that it would not react any more with the "heat precipitin." By immunizing rabbits with an "heat-alkali"-antigen, however, antisera are obtained which are capable of reacting strongly with such insoluble, NaOH-treated serum-proteins.

A prolonged immunization course is required, as after the first 3-5 injections of this antigen, the rabbit serum usually will react only with heated and not with heat-alkali-denatured serum-proteins, and acquires the latter property after further injections. The antisera described in this paper were the product of 6 injections, and were able to react both with heated only and with heat-alkali-denatured serum-proteins.

Our observations agree with those of Rosenberg (1926) that heat-alkali antisera are able to precipitate normal blood sera, but their titres against this antigen was in our experiments very much lower than against heated sera. Incidentally, Schmidt's (1912) heat-alkali-antisera failed to react with normal sera at all.

All the antisera produced in this work were non-specific and gave cross reactions with heterologous normal and heated serum-antigens. This evidently was the result of the multiple inoculation technique. However, it is possible to restore specificity by absorbing the cross-reacting "heat"-antibodies with heated antigens. The antigen for this purpose must be heated at least at 80°C. to absorb the corresponding heterologous "heat"-antibodies. There is evidence that an antiserum may contain a complex of various "heat"-antibodies. The absorption, for example, of antibodies which react only with antigens heated at 70°C. does not eliminate those antibodies which might react with antigens heated at higher temperature. Only absorption with an antigen of the 80-degree type eliminates all "heat"-antibodies which might react with antigens of the

70-100°C. range. Absorption with heated antigens, however, does not remove the "normal" antibodies though some changes in the normal antibody titres are expected after the absorption of "heat"-antibodies.

Absorption experiments have shown also that heated heterologous antigens can remove, in addition to the corresponding antibody, some other heterologous antibodies, and exceptionally also the homologous antibodies when three different antigens are used for absorption simultaneously. Normally, however, two heterologous antigens are required in a certain combination to absorb from each antiserum all the heterologous antibodies.

The phenomenon of disappearance during absorption of corresponding and some related heterologous normal-antibodies is lengthily discussed by Weitz (1952) on the basis of qualitative and quantitative differences in the antigenic components. The disappearance of unrelated heterologous "heat"-antibodies, however, by absorption with heated antigens might indicate that heat to a certain extent destroys or alters the species character of individual antigens, as a consequence of which an antigen is able to absorb other species antibodies.

Though absorption of cross-reacting "heat"-antibodies is certainly possible, it unfortunately renders the antiserum largely valueless against NaOH-extracted insoluble serum-proteins as only two of the four antisera retained their capacity for homologous reaction. Unabsorbed antisera appear more satisfactory in this respect, but the likelihood of cross-reactions has to be considered, though three of the four unabsorbed (non-specific) antisera surprisingly acted quite specifically.

The findings of Rosenberg (1926) that heat-alkali-antisera are suitable for differentiating cooked meats could not be sustained, since none of our antisera, absorbed or not, gave even a slight precipitin reaction with saline-extracted raw or cooked meat or with NaOH-extracted insoluble (powdered) meat-proteins. Even boiling the meat up to 3 hours, which, according to Rosenberg (1926) enhances the release of insoluble protein into the solution and consequently also the success of a precipitin reaction, failed completely. This failure of our antisera to give reactions with meats is not readily explainable, since some of our antisera seemed to have even higher "heat"-antibody titres than Rosenberg's sera. It is possible that perhaps the blood content in the meat samples (Tammemagi 1954b) has some bearing on these discrepant results. Another explanation might be that our neutralization of the NaOH-extracted protein to pH 7·5 with HCl was not the same degree as Rosenberg's, where phenolphthalein was used as an indicator. Underneutralization might leave the protein solution too alkaline and may prevent precipitation, and so lead to false results. However, ring-precipitation appear quite distinctly in our experiments with NaOH-extracted serum-protein solutions which were also neutralized to pH 7·5.

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PINEAPPLE SCALE INVESTIGATIONS.

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SUMMARY.

Studies on seasonal breeding and control of Diaspis bromeliae **(Kerner) were conducted at Rochedale, near Brisbane.**

Throughout a period of 18 months the percentage of living mature females was generally low. The peak occurred in summer and a secondary peak in early winter.

Egglaying by more than 20% of living females occurred in every month except one, with peaks in summer and winter.

A white spraying oil-nicotine sulphate mixture (16-1-640) as single applications at intervals through the year did not consistently reduce the living-insect percentage. Parathion emulsion (0.1%) gave better results than dispersible BHC (0.1%) and chlordane emulsion (0.5%). Parathion (0.025%) as single and double applications combined with thinning of plants did not reveal any early advantages and populations were too low for the differences shown later to be reliable.

The percentage of living insects and the percentage of insects dead from natural causes were generally higher in leaf bases than on leaf laminae; they were not affected by applications of oil-nicotine sulphate. Thinning of plants did not appear to alter the percentage of living insects.

None of the chemicals used influenced flowering of pineapple plants, but technical and deodorised BHC as aerial applications five or more weeks before harvesting tainted fruit.

Parasitism by Aphytis **sp. amounted to a mean of 16.4% of total insects, while predators, mainly ladybirds, which were markedly more active on leaf laminae than in leaf bases, accounted for a mean of 22.8%. The effect of the oil-nicotine sulphate on both parasites and predators was negligible.**

I. INTRODUCTION.

The pineapple scale (*Diaspis bromeliae* (Kerner)) was described from Europe and has been recorded from pineapple and related plants in many countries. Turner (1891, p. 84) in New South Wales stated "the only insects . . . . . seen preying upon the pineapple are the Mealy Bug and the Scale," but gave no indication of the locality. Froggatt (1914, p. 880) remarked that the insect "is doubtfully identified from Queensland." Tryon (1928, p. 34) reported that "This insect was to be met with in the Brisbane area infesting plants of the pineapple family (Bromeliaceae). Fortunately it has spontaneously disappeared."

In 1942 the insect was found heavily infesting a few commercial plantations in the Rochedale district near Brisbane (Jarvis 1944). Light infestations continued for several years but in the second half of 1948 populations increased and, apparently accompanying the post-war expansion of pineapple growing, the insect became dispersed. It was then located in a number

Fig. 1.

Pineapple Scale Infestation on Fruit and Top.

of plantations in the Rochedale district, in several areas in adjoining districts and in two plantations further afield. Another infested plantation outside the Rochedale district was recorded in February 1955. This and some of the other instances of spread were traced to planting material from Rochedale.

Infestations have occurred in plantations of both rough-leaved and smooth-leaved varieties of the pineapple (*Ananas sativa* (L.) Merr.), and in two Brisbane parks on *Billbergia* spp.

II. SEASONAL BREEDING.

Studies of seasonal breeding were made by taking regular samples from 10 sites in a 5-acre plantation of 5-year-old rough-leaved pineapples. Initially two moderately infested leaves were selected from each site. On these 25 mature females, taken at random, were determined as either living or dead, and the living were examined for evidence of egglaying. When sampling commenced in May 1950 insects were numerous and full counts were possible on 10 leaves. Populations gradually declined until more than 120 leaves were required to provide 250 mature insects. After 18 months, populations were so low that counts were discontinued.

Generally the percentage of living insects was low (Fig. 2), and exceeded 50 in only 5 of the 18 months. The peak occurred in summer and a secondary one in early winter.

Egglaying occurred in every month of the year and except for January 1951 the percentage of living insects with eggs was consistently over 20 (Fig. 2). Peaks were shown in both summer and winter. Furthermore, observations made in connection with the oil-nicotine sulphate control trial, dealt with later, revealed extensive egglaying in the early winter of 1949.

III. CONTROL TRIALS.

Three control trials and a fruit taint trial were undertaken. The first control trial was mainly for the purpose of checking the value of the oil-nicotine sulphate mixture in use by Rochedale growers, the second to evaluate parathion, BHC and chlordane, and the third as a further test with parathion and to determine the influence of plant density on chemical control.

(1) First Control Trial.

Method.

This trial consisted of duplicated blocks in 3 acres of 4½-year-old rough-leaved pineapples (Fig. 3). The treatment comprised 16 parts of white spraying oil (80% mineral oil), 1 part of nicotine sulphate (40% nicotine) in 640 parts of water. This was applied in June and December 1949 and March 1950 by a power sprayer at 150 lb. pressure and 150 gal. per acre. The spray filled to capacity all leaf bases but in some places the density of plants prevented complete cover on all leaves.

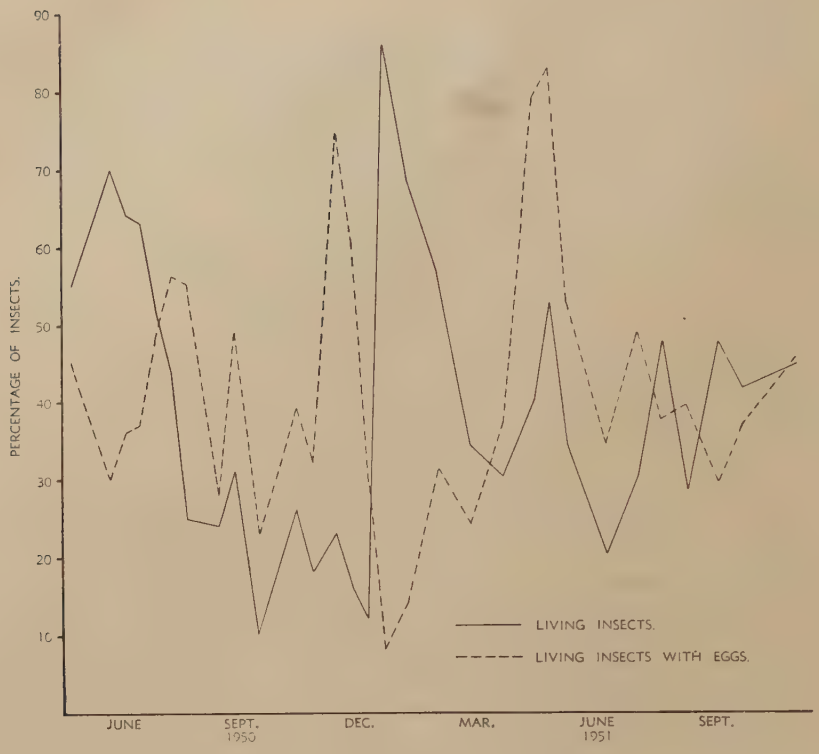

Fig. 2.

Percentages of Living Insects, and Insects With Eggs.

Pretreatment and post-treatment counts of insects were made for each of the three applications. Leaves representing two leaf laminae and two leaf bases were taken from 10 positions of moderate infestation in each block and on these 25 consecutively placed mature females were determined as living, dead or parasitised. Later counts included insects killed by predators.

Discussion.

In June 1949, the percentage of living insects in the untreated areas was 64·2 (Fig. 4). This showed an early decline and then a gradual downward trend continued until July 1950 when only 9·4% of the insects were alive and populations were low.

In the treated areas the percentage of living insects in June 1949 was 57·4 (Fig. 4). The first application of oil-nicotine sulphate had an immediate slight benefit which was still evident in July. The second application made in December did not have any apparent beneficial effect, but this may have been associated in some way with increased activity of natural enemies, which are discussed later. A third application, in March 1950, like the first seemed to exercise a slight insect kill but the effect had diminished by the following July. Except for the initial count, the percentage of insects dead from causes other

Fig. 3.

Pineapple Plants in First Control Trial.

than natural enemies was practically uniform in the untreated areas (Figs. 4 and 6), and other than for slightly increased differences following the June and March applications, the results for both treated and untreated areas were more or less parallel. Single applications of oil-nicotine sulphate, although repeated, were therefore of little economic value.

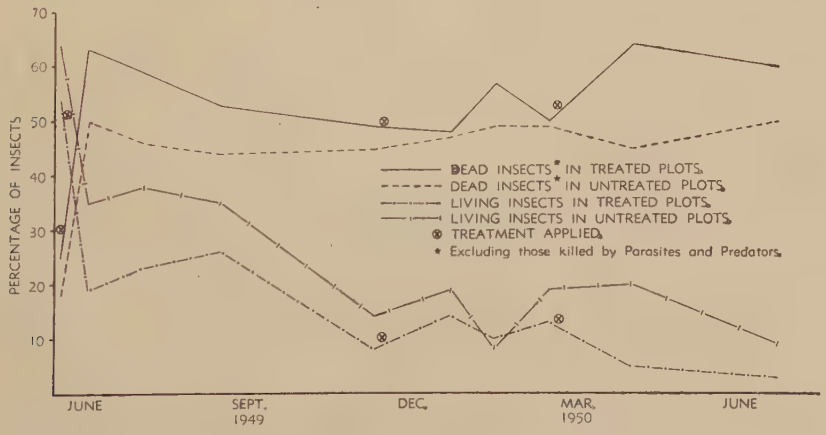

Fig. 4.

Percentages of Living and Dead Insects in Treated and Untreated Plots.

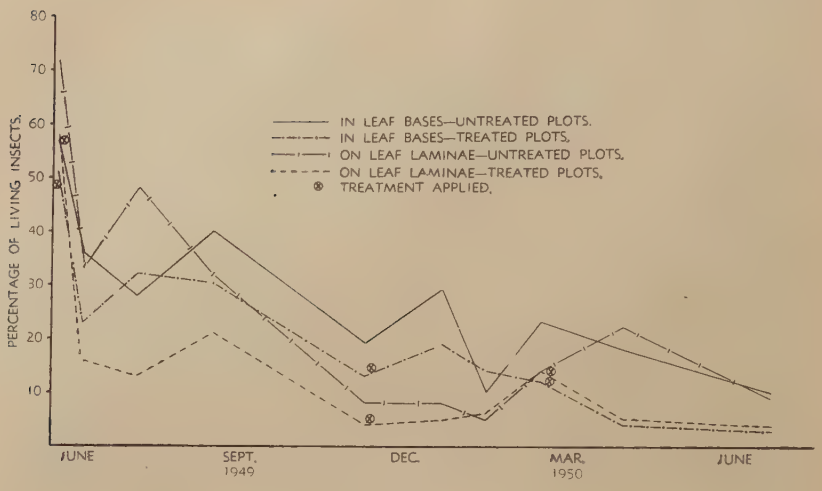

Fig. 5.

Percentages of Living Insects in Leaf Bases and on Leaf Laminae in Untreated and Treated Plots.

The greater proportion of insects occurred on the upper leaf surface within the protection of the ensheathing leaf bases and the data showed that the percentage of living insects and the percentage of dead insects except those killed by natural enemies were greater in this position than on leaf laminae (Fig. 5). Furthermore, the spray applications caused a slightly greater kill of insects in leaf bases than on the laminae (Fig. 6).

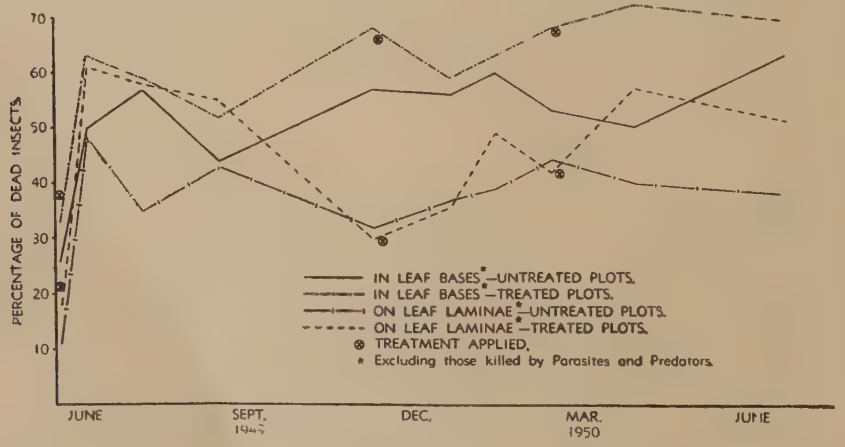

Fig. 6.

Percentages of Dead Insects in Leaf Bases and on Leaf Laminae of Untreated and Treated Plots.

(2) Second Control Trial.

Method.

This trial covered part of three acres of 18-months-old smooth-leaved pineapples (Fig. 7). The area was divided into three sites and on each five treatments were arranged in a latin square, unit plot being five yards of single-plant row. Treatments were as follows.—(*a*) oil-nicotine sulphate (16-1-640); (*b*) chlordane emulsion (0·5%); (*c*) untreated; (*d*) dispersible powder BHC (0·5% gamma isomer); (*e*) parathion emulsion (0·1%).

Fig. 7.

Pineapple Plants in Second Control Trial.

Single applications were made in August and December 1949 with a knapsack and bucket pump respectively, using on each occasion 188 gal. per acre for each treatment. The spray was first directed along the sides of the row of plants and finally over the top. The side application covered the lower leaf surfaces and filled the lower leaf bases, while the top application covered the upper leaf surfaces and filled the throat of the plants.

At each sampling 10 leaves were taken from each plot, five from each side of the row. The initial samples were taken prior to the first application in August 1950 and subsequent samples approximately at monthly intervals.

Discussion.

The anticipated rise in populations did not occur. In fact, populations generally decreased, and did so to such an extent over two of the sites that

results of insect counts from these were of no value; therefore only one site is included in the following discussion.

The insects in this area were almost entirely in the leaf bases and parasites and predators were practically absent. Counts were made for living and dead only, and comparisons were made directly between total numbers of insects and percentages of dead.

The number of insects in the untreated plots (Fig. 8) increased from 89 in August 1950 to 337 in September. In November it had decreased to less than 150 and by the following February the expected extensive summer breeding provided little increase. The numbers in the several treated plots (Fig. 8) showed the same general trends but the decline in comparison with the untreated plots was particularly noticeable after the December application. The oil-nicotine

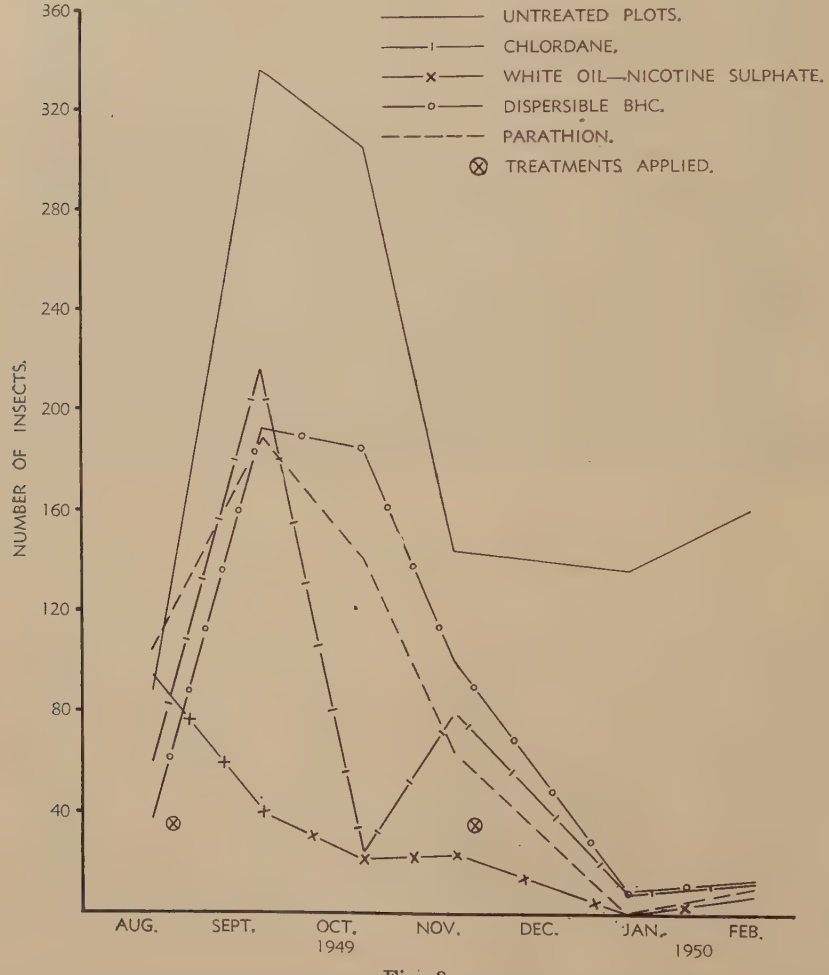

Fig. 8.

Numbers of Insects in Treated and Untreated Plots.

sulphate treatment appeared to give an immediate marked reduction not shown by the other treatments, but finally the numbers of insects present were comparable for all treatments and averaged 6·2% of the number in the untreated plots.

The percentage of dead insects (Fig. 9) increased slightly after the first sampling in the untreated plots, then decreased gradually until November. From January to February an increase was again shown, making the dead-insect percentage comparable to that of the previous September. All spray treatments caused an early marked increase in the percentage of dead insects

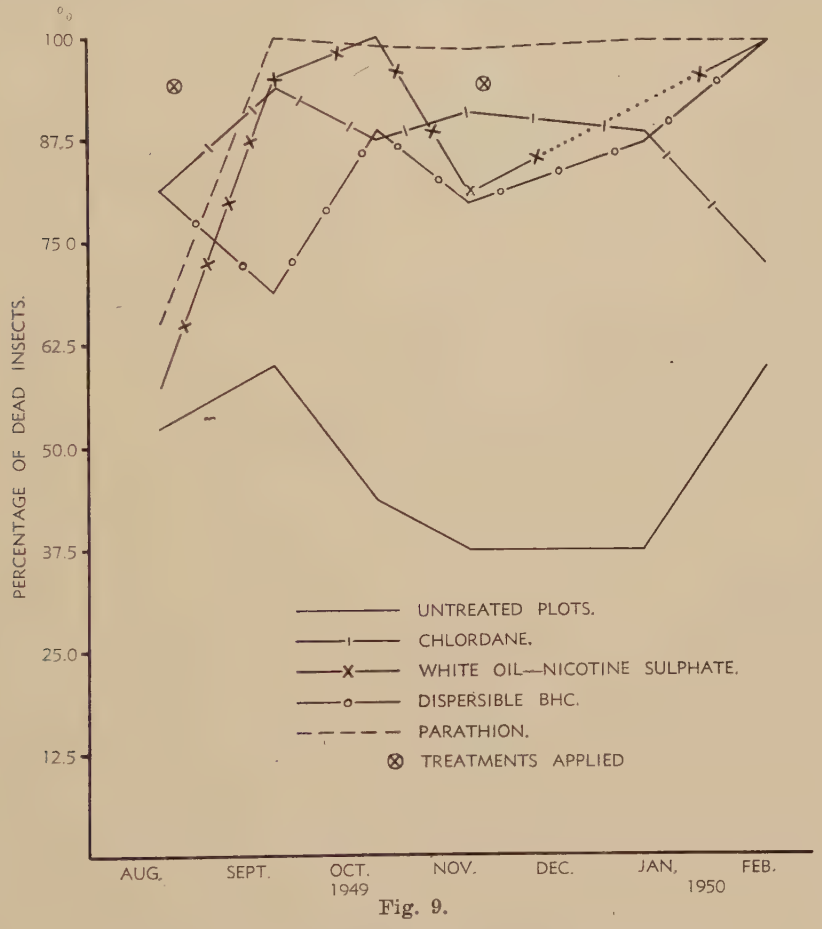

Fig. 9.

Percentages of Dead Insects in Treated and Untreated Plots.

except the dispersible BHC. Parathion maintained throughout the remainder of the trial a high dead-insect percentage. BHC and oil-nicotine sulphate varied, but again gave a marked increase after the second application and at the final sampling all insects were dead. Chlordane also varied and finally showed a downward trend.

Counts of the number of parent plants with suckers, flowers and fruit were made for each site at the commencement and a month before completion of the trial to determine whether any treatments influenced flowering. An analysis of the counts of flowers and fruit is given in Table 1.

Table 1.

INCREASES OF FLOWERS AND FRUIT EXPRESSED AS PERCENTAGES.

Treatment.	Transformed Values.				Equivalent Percentage Increase.			
	Sites.			Mean.	Sites.			Mean.
	1	2	3		1	2	3	
Oil-nicotine sulphate	28·4	31·8	24·4	28·2	22·7	27·8	17·0	22·4
Chlordane	41·6	29·8	23·5	31·6	44·1	24·7	15·9	27·5
Untreated	27·1	30·9	20·0	26·0	20·8	26·4	11·7	19·3
Disp. BHC	38·4	36·6	30·3	35·1	38·6	35·5	25·4	33·0
Parathion	34·7	30·7	29·3	31·6	32·4	26·0	23·9	27·4
Means	34·1	32·0	25·5	30·5	31·4	28·0	18·5	25·8
S.E.	±5·01			±2·89	..	..	..	..

Differences are not significant.

The BHC treatment was responsible for slight injury to young leaves but not enough to affect their growth.

(3) Third Control Trial.

Method.

The area in this trial comprised part of three acres of 5½-year-old rough-leaved pineapples and consisted of four rows each 75 yards long of densely growing plants. Six treatments were randomised in four replicates, unit plot being 10 yards of double row. The treatments were no application, and single and double applications of parathion on thinned and unthinned plants. The strength of the spray was 0·025% and where double applications were made a two weeks' interval intervened. Applications were made in June and December 1950 by means of a bucket pump and averaged 250 gal. per acre on the unthinned plants and 180 gal. on the thinned plants, an endeavour being made to cover all leaf surfaces and to fill the leaf bases and throats of all plants.

The sampling in this trial initially comprised 20 infested leaves from each plot, 10 from each side of the row. On these, living and dead were recorded from a total of 250 scales, not exceeding 15 on any one leaf.

To determine whether the number of leaves taken represented a sufficient sample, an analysis using a transformed variate was made of the counts of living insects from the pretreatment samples. Taking into consideration

variations from leaf to leaf and from plot to plot it was possible to determine the necessary differences for significance of various numbers of leaves combined with different numbers of replicates. These differences are given in Table 2.

Table 2.

DIFFERENCES FOR SIGNIFICANCE IN COUNTS OF LIVING INSECTS.

Replicates.	Number of Leaves.						
	5	10	15	20	25	30	∞
4	16·0	13·7	12·8	12·3	12·1	11·9	10·8
6	13·1	11·2	10·5	10·1	9·8	9·7	8·9
8	11·3	9·7	9·1	8·7	8·5	8·4	7·7
10	10·1	8·7	8·1	7·8	7·6	7·5	6·9

It was shown that replication was more effective for all sizes of sample and that increases beyond 15 leaves per plot provided little improvement in accuracy. The trial had already commenced with four replicates and therefore 15 or more leaves were taken at each sampling.

Discussion.

In July 1950, a month after the applications of parathion, the percentage of living insects dropped for all treatments but was more than twice as high in the respective unsprayed plots as in those sprayed (Fig. 10). In September

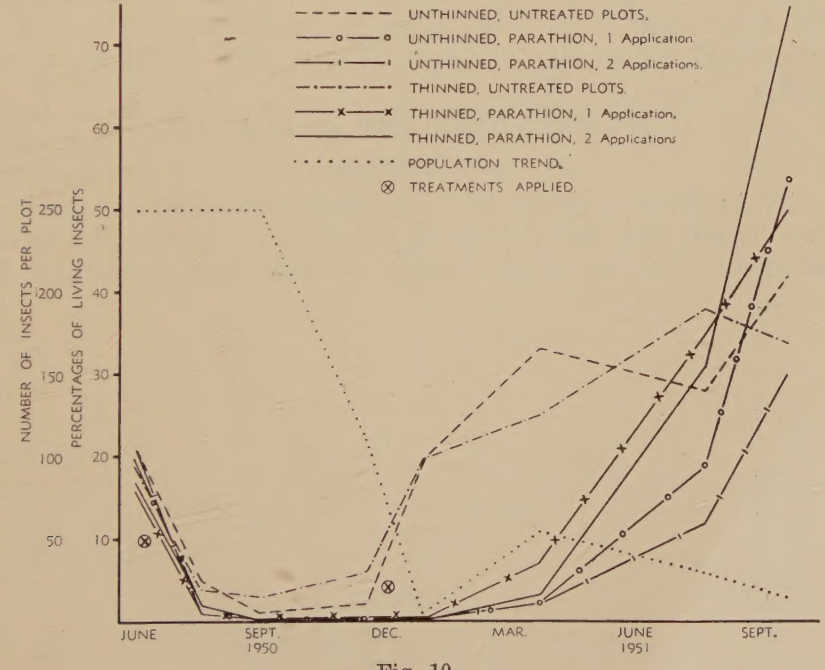

Fig. 10.

Percentages of Living Insects in Treated and Untreated Plots, and Population Trend.

and in November and December no living insects could be found on any sprayed plot. A few living were present in the unsprayed plots in September and this increased markedly in the following four months, but at the same time populations were declining throughout the whole area (Fig. 10). Populations remained so low that further applications were not made and the trial was discontinued in October 1951.

From the counts made, the difference shown between the sprayed and unsprayed plots may be taken to indicate benefit from some of the treatments. Differences between thinned and unthinned plots, however, were not consistent.

(4) BHC Taint Trial.

At Nambour early in 1952 field applications of a dust and a spray of technical BHC and sprays of lindane emulsion and dispersible powder were made on pineapple fruit. The dust contained 0·05% and the sprays 0·033% of gamma isomer. One series of fruit was treated in February and harvested when ripe 7-9 days later, while another series was treated in January with maturity and harvesting extending from 5 to 12 weeks later. Each fruit was submitted to a tasting panel.

No taint was detected in any untreated or treated fruit maturing and harvested up to 5 weeks after treatment, but most of the later maturing fruit were tainted. The younger fruit apparently can readily absorb BHC, whether crude or deodorised, and retain it in the tissue. Maturity and harvesting of pineapple fruit normally extends over a long period and any aerial application of BHC would result in some tainted fruit. As off-flavour or taint in food is not tolerated by the public, aerial application of BHC should not be made to pineapples.

IV. PARASITES AND PREDATORS.

(1) Parasites.

An Aphelinid (*Aphytis* sp.) was consistently active on the pineapple scale and counts were made of the insects killed by this parasite in the first control trial. These are shown in Figs. 11 and 12 as percentages of total

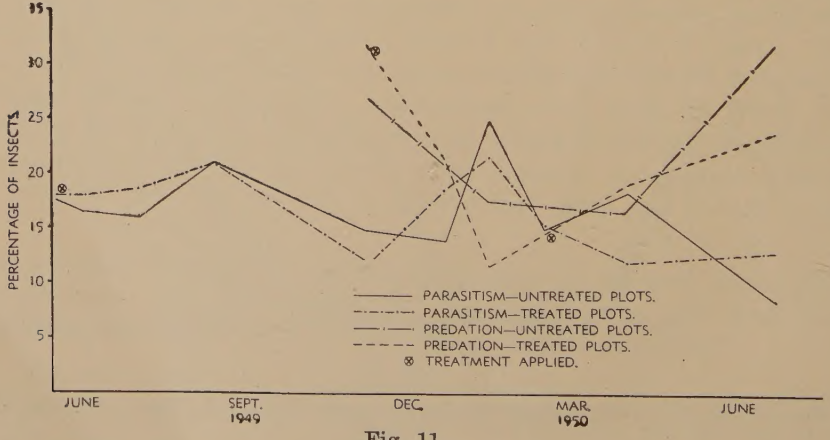

Fig. 11.

Percentages of Insects Killed by Parasites and Predators in Untreated and Treated Plots.

insects. From June 1949 to July 1950 these percentages ranged from 8·4 to 25·2, being high in late winter and late summer.

In the areas treated with oil-nicotine sulphate the percentage of parasitism varied from 11·8 to 21·2. The oil-nicotine sulphate applications therefore had little effect on the work of the parasites.

The differences in parasitism on leaf laminae and in leaf bases were negligible (Fig. 12).

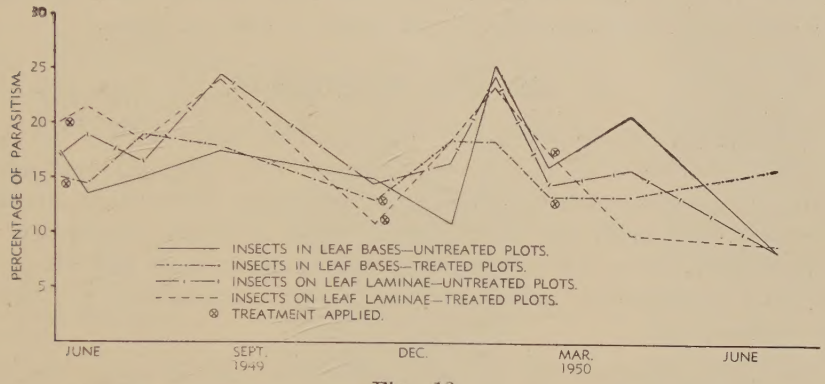

Fig. 12.

Percentage of Parasitism in Leaf Bases and on Leaf Laminae on Untreated and Treated Plots.

(2) Predators.

During the progress of the oil-nicotine sulphate trial it was noticed that predators, mainly ladybirds in the genera *Rhizobius* and *Orcus*, increased as important factors in reducing the percentage of living insects. Consequently, commencing with the November 1949 samples, counts were made of insects

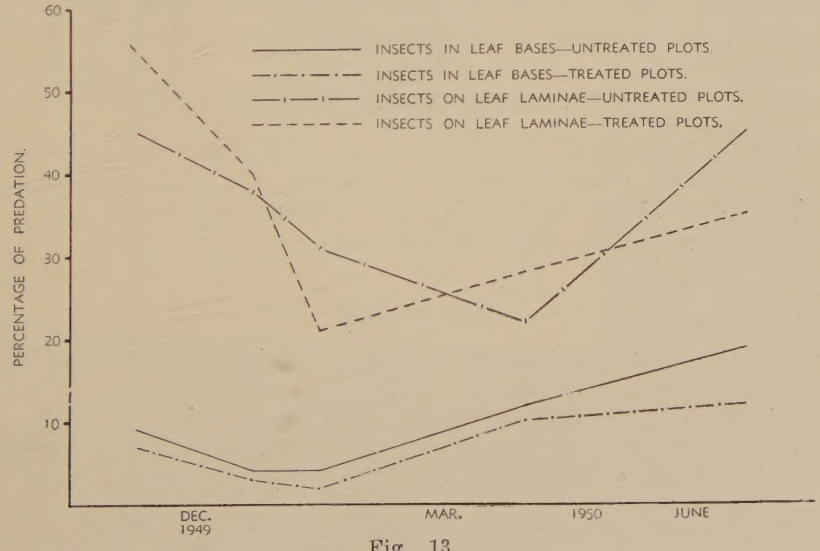

Fig. 13.

Percentage of Predation in Leaf Bases and on Leaf Laminae in Untreated and Treated Plots.

killed by these enemies (Figs. 11 and 13). The data showed that predators accounted for an average of 22·8% of total insects present in the untreated areas and 21·7% in the treated areas. An outstanding feature was the difference between activity on leaf laminae and in leaf bases (Fig. 13). In the untreated areas the percentage of scale insects killed by predators on the leaf laminae ranged from 21·7 to 45·0, and in the leaf bases from 4·0 to 19·0. Similarly, in the treated areas the range was from 21·4 to 55·6% on leaf laminae and from 1·9 to 12·3% in the leaf bases. The figures also showed that the treatment did not have any noticeable effect on the work of the predators.

V. GENERAL DISCUSSION.

Infestations of the pineapple scale in 1948 were severe, but in the second half of 1949, due largely to biological and other natural agencies, populations generally began to decline and then became so low that satisfactory experimental work could not be continued. Such a decline apparently is not unusual, because Tryon, when first recording the insect from this State, found that it had "disappeared," and the severe infestations observed in 1942 later became light.

The records suggest that the pineapple scale is not of major importance. The occasional severe infestations, however, have been sufficient to reduce yields and curtail the economic life of plantations. Such occurrences could have serious repercussions in the industry if the insect were to spread to the large pineapple growing districts.

Control trials have shown that a mixture of white spraying oil and nicotine sulphate (16-1-640) gave only a small kill of insects and dispersible BHC (0·1%) and chlordane emulsion (0·5%) were ineffective. Parathion (0·1%) showed some promise but results from the lower concentration (0·025%) were inconclusive. The problem of chemical control therefore requires further investigation when adequate populations of the insect appear.

VI. ACKNOWLEDGEMENTS.

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